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# Photosynthetic characteristics and effects of exogenous glycine of *Chorispora bungeana* under drought stress

N. YANG[*,+], C.-L. WANG[*], W.-P. HE[*], Y.-Z. QU[*], and Y.-S. LI[*]

*College of Life Science, Northwest Normal University, Lanzhou 730070, China*[*]

## Abstract

We investigated the photosynthetic characteristics of *Chorispora bungeana* under conditions of drought stress caused by different concentrations of polyethylene glycol-6000 (PEG; 0, 5, 20, and 40%) and various concentrations of exogenous glycine (0, 5, 10, and 20 mM) with 20% PEG. We showed that moderate and severe drought stress of PEG reduced the chlorophyll (Chl) content (both Chl *a* and *b*), maximal quantum yield of PSII photochemistry ($F_v/F_m$), actual photochemical efficiency of PSII in light ($Y_{II}$), and quantum yield of regulated energy dissipation ($Y_{NPQ}$), while Chl *a*/*b* and quantum yield of nonregulated energy dissipation ($Y_{NO}$) increased. The low and moderate drought stress increased $Mg^{2+}$ and $Fe^{3+}$ contents, while a decrease in $Mg^{2+}$ and $Fe^{3+}$ was found under severe drought stress. Compared to sole PEG stress, the addition of exogenous 10 mM glycine increased Chl, $Mg^{2+}$ and $Fe^{3+}$ contents, $F_v/F_m$, $Y_{II}$, and $Y_{NPQ}$, and reduced $Y_{NO}$. On the contrary, 20 mM glycine showed an opposite effect, except for $Y_{NO}$. Our results proved that Chl contents and fluorescence parameters are reliable indicators for drought tolerance of *C. bungeana*. We suggest that a proper glycine content can relieve the effect of drought stress on *C. bungeana*.

*Additional key words*: chlorophyll *a*/*b* ratio; electron transport; iron content; magnesium content; nitrogen.

## Introduction

*Chorispora bungeana* is an alpine subnivean herbaceous plant prone to tolerate such abiotic stresses as low temperatures, drought stress, low air pressure, high radiation, and strong winds. The species inhabits freezing thaw tundra with glacial borders at altitudes between 3,800–3,900 m along the Glacier of the Urumqi River in Tianshan Mountains, Xinjiang, China, where most of other flowering plants have great difficulty to grow. Previous studies showed that the species has no special morphological features, but that it still survives under harsh freezing environments (Ayitu *et al.* 1998, Chang *et al.* 2006, Yang *et al.* 2012). According to Chang *et al.* (2006), Fu *et al.* (2006), and Wu *et al.* (2009), the species has been proven to retain some stable variations of physiological and genetic characteristics in response to environmental stresses. There are reports about the molecular and physiological mechanisms of *C. bungeana* under low temperature stress, but very few reports about their physiological mechanisms in response to drought. As a naturally resistant species, *C. bungeana* is an ideal species to determine resistance mechanisms in plants (Guo *et al.* 2006).

Drought is a major environmental factor limiting plant growth and productivity. It is very important to find adaptation mechanisms towards drought in plants. The drought stress impact on the plant photosynthetic machinery has been studied in detail and possible mechanisms have been analyzed (Cornic *et al.* 1996). Studies have shown that agricultural and horticultural crops may be able to take up significant quantities of dissolved organic nitrogen (DON), such as amino acids and some simple proteins (Mo *et al.* 2003, Jones *et al.* 2004, Vieublé-Gonod *et al.* 2006). Scientists have revealed the function of nitrogen from many aspects in plants (Cui 2007). Nitrogen promotes the Chl content of flue-cured tobacco seedlings under drought stress, and improves their photosynthetic potential (Jin *et al.* 2012). Nitrogen may improve drought resistance (Bänziger *et al.* 1999), however, a high nitrogen amount may restrain plant growth.

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[+]Corresponding author; e-mail: xbsd-yn@163.com

*Abbreviations*: Chl – chlorophyll; DON – dissolved organic nitrogen; $F_0'$ – the minimal fluorescence in the light-adapted state; $F_m'$ – maximal fluorescence in the light-adapted state; $F_v'/F_m'$ – the photochemical efficiency of open PSII; HN – high nitrogen; LN – low nitrogen; MN – moderate nitrogen; $q_L$ – coefficient of photochemical quenching (lake model); $q_P$ – coefficient of photochemical quenching (puddle model); SD – standard deviation; $Y_{NO}$ – nonregulated energy dissipation; $Y_{NPQ}$ – quantum yield of regulated energy dissipation; $Y_{II}$ – actual quantum yield of PSII photochemistry; $Y_{PSII}$ - effective quantum yield of PSII photochemistry.

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The Chl fluorescence parameters are closely linked to photosynthesis and are correlated with the rate of carbon exchange (Fracheboud *et al.* 2004, Guo *et al.* 2005). Chl fluorescence analysis is a powerful tool in studying plant's response to environmental stress, remote sensing, and plant phenotyping (Fracheboud *et al.* 2003, Lazár 2015). Chl fluorescence analyses have revealed that drought stress caused considerable injury of photosynthetic apparatus. Some studies showed that actual quantum yield of PSII photochemistry ($Y_{II}$) in cotton plants decreased under drought stress conditions (Ennahli and Earl 2005, Isoda 2005). Similarly, it is concluded that the impairment of PSII is mainly due to a reduction in photochemistry ($q_P$) and less employment of dissipative mechanisms for long-term drought stress in cotton plants (Kitao *et al.* 2007). Glycine is found in higher contents in soil, with a small molecular mass and simple structure. It is the ideal model of a nitrogen source to study plant organic nitrogen (Ge *et al.* 2009). But very little is known about the effect of exogenous glycine on traits, such as Chl content and Chl fluorescence parameters, under drought stress in plants.

In order to understand the photosynthetic characteristics and effects of exogenous glycine under drought stress in *C. bungeana*, we studied changes of the Chl content, Chl fluorescence parameters, and ion utilization under drought stress with different contents of exogenous glycine. The relationship of these traits, drought tolerance, and glycine content were also discussed in this study.

## Materials and methods

**Plant material and growth conditions**: *Chorispora bungeana* seedlings *in vitro* were obtained according to the Yang's method (2012) with some modification. The seed coats were removed with sand paper, soaked in 70% ethanol for 30 s and then soaked in 0.1% mercuric chloride solution for 6–8 min ( it was done for surface sterilization), and lastly washed with sterile water three times. Cotyledons were then cut off and inoculated in the culture medium without any type of plant growth hormone. When the regenerated seedlings were approximately 4 cm tall, they were transferred to the Murashige and Skoog (MS) medium containing 1 mg $l^{-1}$ of 6-benzylaminopurine (6-BA) and 3% sucrose, and cultured at 25°C under 16 h photoperiod [irradiance of 80 μmol(photon) $m^{-2}$ $s^{-1}$].

**Drought stress and glycine treatment**: The methods of different PEG-6000 and glycine treatments were carried out according to Yang *et al.* (2015) with some modification. After being three times subcultured, *C. bungeana* seedlings with high vigor and of the same size were chosen to grow with 5, 20, and 40% of PEG-6000 on solid-liquid medium for 72 h. The leaves were collected at different time points (6, 12, 24, 48, and 72 h) from different drought treatments. In order to study the effect of exogenous nitrogen on *C. bungeana* seedlings, glycine (5, 10, and 20 mM, further as LN, MN, and HN, respectively) was added to the liquid culture medium with 20% PEG-6000.

| Culture medium | Drought stress (PEG-6000 [%]) | Exogenous glycine [mM] + 20% PEG-6000 | MS | Agar | 6-BA |
|---|---|---|---|---|---|
| Solid | - | - | + | + | + |
| Liquid | 0 | 0 | + | - | + |
| | 5 | 5 | + | - | + |
| | 20 | 10 | + | - | + |
| | 40 | 20 | + | - | + |

**Determination of Chl**: Chl was extracted with 80% acetone and determined according to Porra *et al.* (1989). The Chl content in the supernatant after centrifugation (1,000 × *g* for 10 min) was estimated by reading the absorbance at 645 and 663 nm with a UV spectrophotometer (*UV-120-02*, *Shimadzu*, Kyoto, Japan).

**Iron and magnesium content determination**: A solid-liquid medium supplemented with 0, 20, 40% PEG-6000 and 0, 5, 10, and 20 mM glycine were taken and acidified with $HNO_3$ (1%, v/v) and the actual concentrations of $Fe^{3+}$ and $Mg^{2+}$ were determined using a flame atomic absorption spectrophotometer (*WFX210*, *Beijing Beifeng-Ruili Analytical Instrument (Group) Co. Ltd.*, China). The leaves of *C. bungeana* seedlings were prepared for $Fe^{3+}$ and $Mg^{2+}$ measurement according to Achary *et al.* (2012) with some modification. The leaves were thoroughly washed with deionized water and dried to a constant mass at 80°C. The dry sample was dissolved in a solution containing nitric acid ($HNO_3$), and digested in a closed microwave digestion system (*Multiwave 3000*, *Anton Paar*, Austria) for 2.5 h, and then evaporated till dryness. The resulting ash residue was dissolved in $HNO_3$ (1%, v/v), and total $Fe^{3+}$ and $Mg^{2+}$ contents were measured with the above mentioned spectrophotometer.

**Chl fluorescence measurements**: Chl fluorescence is mostly measured using the pulse amplitude modulation (PAM) technique. Chl fluorescence in the leaves of *C. bungeana* seedlings was measured at room temperature and at an ambient $CO_2$ concentration using a portable fluorometer (*PAM-2000*, *Walz*, Germany) after dark treatment for 30 min. The fluorometer was connected to a leaf-clip holder (*2030-B*, *Walz*, Germany) with a trifurcated fiberoptic (*2010-F*, *Walz*, Germany) and to a computer with data acquisition software (*DA-2000*, *Walz*, Germany). Fluorescence nomenclature was used according to Demmig-Adams *et al.* (1996). Measuring flashes only or flashes and saturating pulse under a dark-adapted state were used to obtain minimal fluorescence for the dark-adapted state ($F_0$) and maximal fluorescence for the dark-adapted state ($F_m$). After that the leaves were continuously exposed to white actinic light. A second saturating pulse at 8,000 μmol(photon) $m^{-2}$ $s^{-1}$ was supposed to determine maximal fluorescence in the light-adapted state ($F_m'$). The actinic light was removed and the minimal fluorescence in the light-adapted state ($F_0'$) was defined by illuminating the leaf with 3 s far-red light [6 μmol(photon) $m^{-2}$ $s^{-1}$]. We selected no less than five leaves from each *C. bungeana* seedling to measure Chl fluorescence according to above principle. There were three *C. bungeana* seedlings for an experiment, and we did three independent measurements. The following parameters were calculated by the *PAM WIN* Data Acquisition System (Krall *et al.* 1992, Lazár 2015) (*Walz*, Effeltrich, Germany): (*1*) maximal quantum yield of PSII photochemistry [$F_v/F_m = (F - F_0)/F_m$], (*2*) actual quantum yield of PSII photochemistry ($Y_{II} = q_p Y_{PSII}$), (*3*) quantum yield of regulated energy dissipation ($Y_{NPQ} = 1 - Y_{II} - Y_{NO}$), (*4*) nonregulated energy dissipation [$Y_{NO} = 1/NPQ + 1 + q_L(F_m/F_0) - 1$], and (*5*) maximal quantum yield of PSII photochemistry for light-adapted state [$Y_{PSII} = F_v'/F_m'$].

**Statistical analysis**: All the data were obtained by an average of at least three replicates. The values shown in the figures were mean values ± SD. Statistical comparisons were carried out by using *SPSS 17.0* software, and significant differences were indicated by different letters ($P<0.05$).

## Results

**Chl *a* and *b* contents** sharply decreased in *C. bungeana* seedlings under moderate and severe drought stress. Compared with the control, contents of Chl decreased 1.00-, 1.24-, 1.37- , 1.21-, and 1.07-fold at different time points (6, 12, 24, 48, and 72 h) under severe drought stress, respectively (Fig. 1*A*). The Chl *a*/*b* ratio was higher than the control one, while the Chl *b* content was always lower than Chl *a* (Fig. 1*B*–*D*).

**Iron and magnesium ions under drought stress**: The actual contents of $Fe^{3+}$ and $Mg^{2+}$ showed a similar change (Fig. 2). The $Mg^{2+}$ content in the leaves was elevated significantly with the increase in PEG-6000 concentration, being higher by 11.5, 54.5, and 46.9% in 5, 20, and 40% of PEG-6000, respectively, than that of the control at 24 h.

The actual $Fe^{3+}$ content in the leaves was significantly elevated to 202, 585, and 130% of the control at 24 h under different concentrations of PEG-6000, respectively. But $Fe^{3+}$ and $Mg^{2+}$ concentrations decreased by 40% PEG-6000.

**Chl fluorescence parameters under drought stress**: $F_v/F_m$ and $Y_{II}$ are shown in Fig. 3*A*,*B*. In the early stages of drought, the $F_v/F_m$ and $Y_{II}$ values showed continuous decline compared with the control. The $Y_{II}$ parameters decreased and were significantly lower than the control. These parameters were significantly affected by both moderate and severe drought stress. Compared with the control, the minimum values in all treatments were observed under the most intense drought stress. The $F_v/F_m$ values were reduced by 52.4, 64.2, 70.2, 79.4, and 70.9% and the $Y_{II}$ values were reduced by 18.6, 28.3, 29.8, 30.2, and 27.1% as compared to the control at different time points (6, 12, 24, 48, and 72h) under 40% PEG-6000 stress, respectively. The $Y_{NPQ}$ and the $Y_{NO}$ values showed different trends. The $Y_{NPQ}$ continued to decline and the $Y_{NO}$ showed a slight increase (Fig. 3*C*,*D*).

**Chl under exogenous glycine and drought stress**: The leaves of *C. bungeana* showed a significant increase in the Chl content, Chl *a*, and Chl *a*/*b* at LN and MN glycine treatments (Fig. 4*A*,*B*,*D*). The Chl *b* content increased significantly in the HN plants (Fig. 4*C*).

**Iron and magnesium ions under exogenous glycine and drought stress**: The leaves of *C. bungeana* showed higher $Fe^{3+}$ and $Mg^{2+}$ contents compared with the control under the increasing glycine concentration and under drought stress (Fig. 5). The $Fe^{3+}$ and $Mg^{2+}$ contents continued to increase in the LN and MN treatments to a maximum which was by 212.2% higher than that of the control at 24 h in MN, and then decreased significantly. Compared to the LN and MN plants, the content of $Fe^{3+}$ and $Mg^{2+}$ significantly decreased in the HN plants (Fig. 5).

**Chl fluorescence parameters** including $F_v/F_m$, $Y_{II}$, and $Y_{NPQ}$ under exogenous glycine and drought stress exhibited the higher values and the differences were apparent in different exogenous glycine treatments comparing with the control. The peaks of these parameters appeared at different times in the MH plants. $F_v/F_m$ increased to 49.7% at 12 h and $Y_{II}$ increased to 146.7% at 48 h (Fig. 6*A*,*B*).

Changes in the $Y_{NPQ}$ and $Y_{NO}$ responded to glycine. Exogenous nitrogen caused a decrease of $Y_{NO}$, and an increase in $Y_{NPQ}$. It was interesting that these parameters were greatly affected by exogenous 10 mM glycine under drought stress (Fig. 6*C*,*D*).

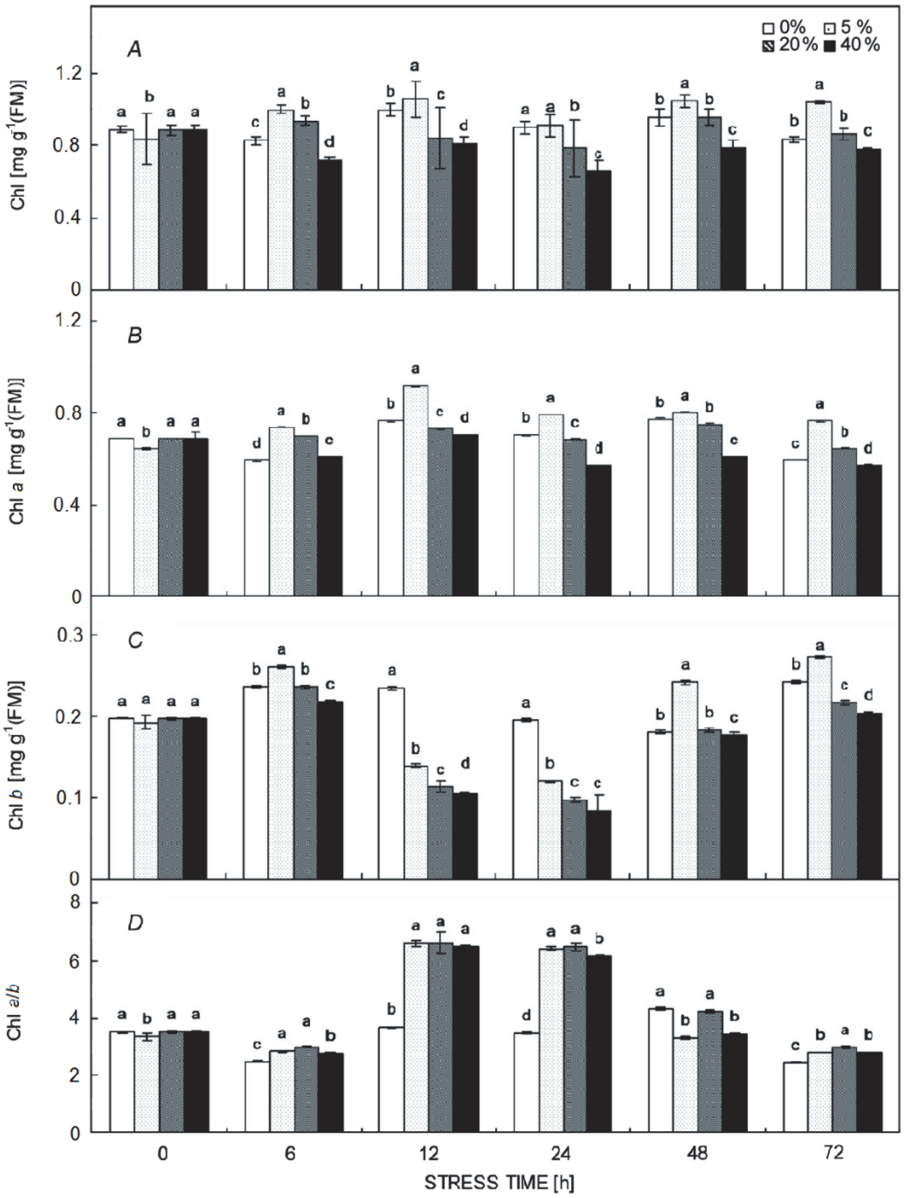

Fig. 1. Effect of drought stress on the content of Chl (*a*+*b*) (*A*), Chl *a* (*B*), Chl *b* (*C*), and Chl *a*/*b* (*D*) in *Chorispora bungeana* plantlets *in vitro* exposed to 0, 5, 20, and 40% PEG-6000 for 72 h. Values are the mean ± SD of at least three independent experiments. Chl – chlorophyll; FM – fresh mass; SD – standard deviation.

## Discussion

**Chl under drought stress**: The Chl content correlated positively with the photosynthetic rate. Studies showed that the high Chl content contributes to an improved yield and transpiration efficiency under drought conditions (Borrell *et al*. 2000, Haussmann *et al*. 2002, Verma *et al*. 2004). The Chl *a*/*b* ratio has been used to evaluate the drought resistance of plants (Flexas *et al*. 2002). Our study showed that the total Chl content, Chl *a*, *b*, and *a*/*b* significantly decreased under moderate and severe drought stress, which illustrated that the Chl synthesis has been inhibited and chlorophyllase activity has increased, thus the Chl decomposition rate increased (Horn *et al*. 2002).

Nitrogen is one of the essential elements for the growth of plants. The content of photosynthetic pigments and the activities of Rubisco are affected by leaf nitrogen concentration (Guan *et al*. 2015). Some reports indicated that amino acids taken up by plants is assimilated in the root and is deaminized in the leaves. Meanwhile, Yamagata *et al*. (1999) also found that plants take up organic nitrogen easily as amidonitrogen and amino acids. In this study, LN and MN treatments with glycine induced a significant increase in Chl synthesis, therefore we speculated a certain concentration of glycine might relieve the effect of drought stress on *C. bungeana* (Ashraf *et al*. 2007).

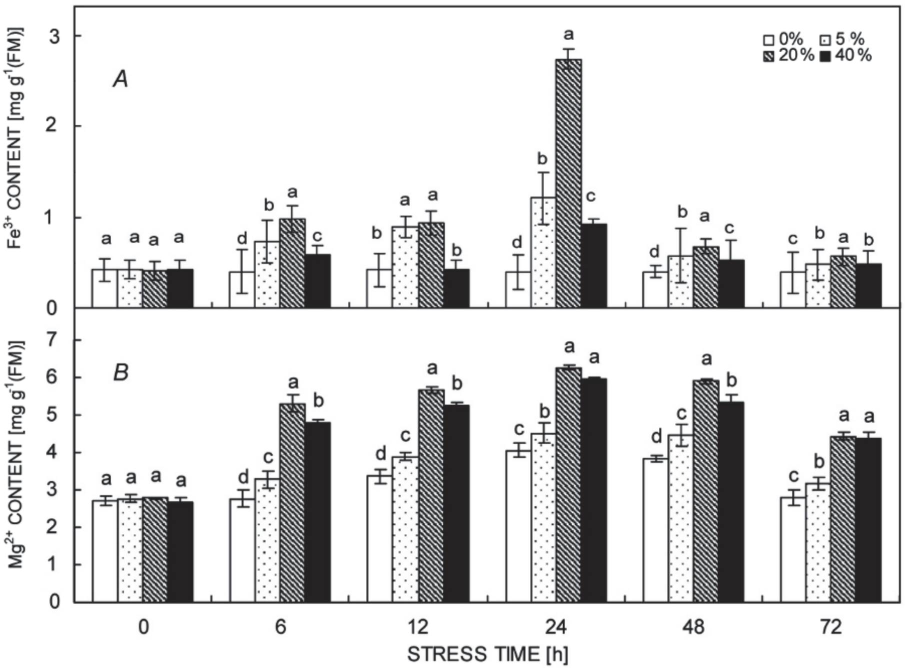

Fig. 2. Effect of drought stress on the content of $Fe^{3+}$ (*A*) and $Mg^{2+}$ (*B*) in *Chorispora bungeana* plantlets *in vitro* exposed to 0, 5, 20, and 40% PEG-6000 for 72 h. Values are the mean ± SD of at least three independent experiments. FM – fresh mass. SD – standard deviation.

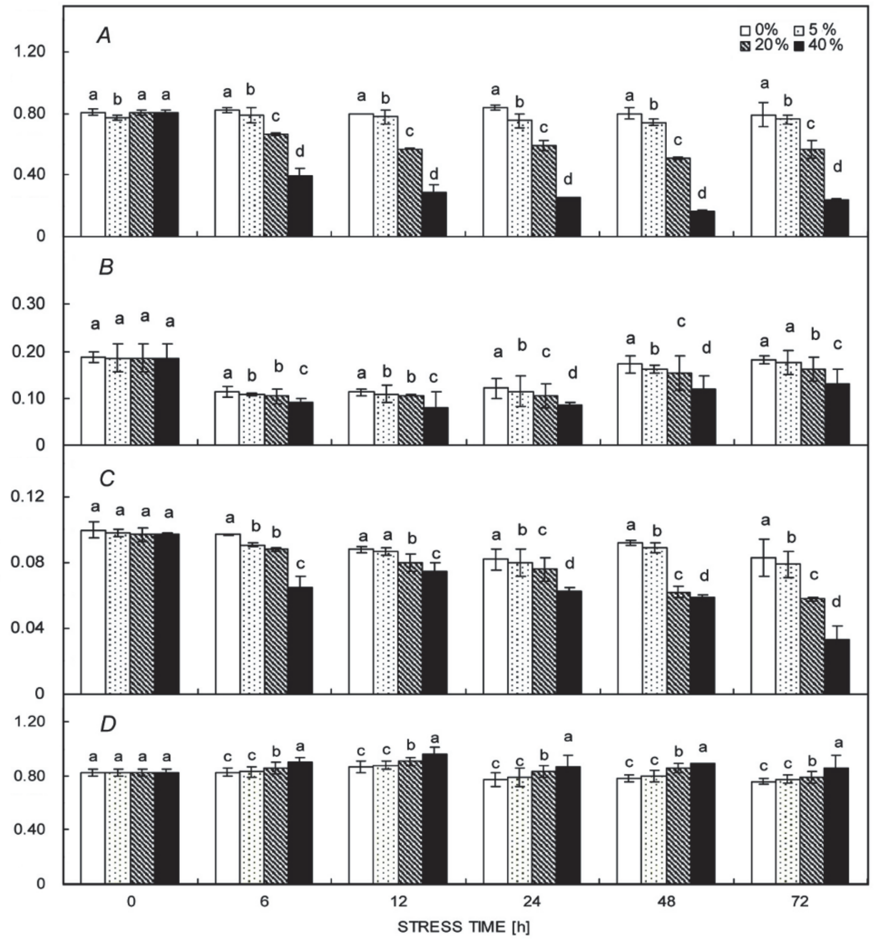

Fig. 3. Effect of drought stress on the content of $F_v/F_m$ (*A*), $Y_{II}$ (*B*), $Y_{NPQ}$ (*C*), and $Y_{NO}$ (*D*) in *Chorispora bungeana* seedlings *in vitro* exposed to 0, 5, 20, and 40% PEG-6000 for 72 h. Values are the mean ± SD of at least three independent experiments. $F_v/F_m$ – maximal quantum yield of PSII photochemistry; $Y_{II}$ – actual quantum yield of PSII photochemistry; $Y_{NPQ}$ – quantum yield of regulated energy dissipation; $Y_{NO}$ – quantum yield of nonregulated energy dissipation; SD – standard deviation.

However, our study also showed that the Chl content decreased under the high concentration of glycine, thus, we suggest that a high concentration of glycine was not beneficial to the synthesis of Chl and relieving drought impact.

**Iron and magnesium ions**: Magnesium, as the important component of the chloroplast, plays an important role in photosynthesis. Reports show that magnesium is also involved in the activation process of phosphoric acid. Besides, it could raise the carbon dioxide affinity for Rubisco, which promotes photosynthesis (Martins *et al.* 2015). Although iron is not the main component of the chloroplast, it still plays a vital role in the synthesis of Chl precursors. The activity of PSII complexes depressed, and the electron transport chain was blocked by iron deficiency (Hulsebosch *et al.* 1996, Sandmann *et al.* 1983). Therefore, understanding the changes of magnesium and iron ions in plant are of significance for studying the photosynthetic activity. Our results demonstrated that an increase of magnesium and iron ion contents might affect the related enzymes of Chl biosynthesis, and enhance its photosynthetic rate in order to adapt towards drought stress. However, the magnesium and iron ion contents were reduced under severe stress, which probably indicated inhibition of Chl synthesis. In this study, exogenous glycine promoted magnesium and iron ions under drought stress, which affected the photosynthesis-related enzymes to elevate photosynthetic efficiency.

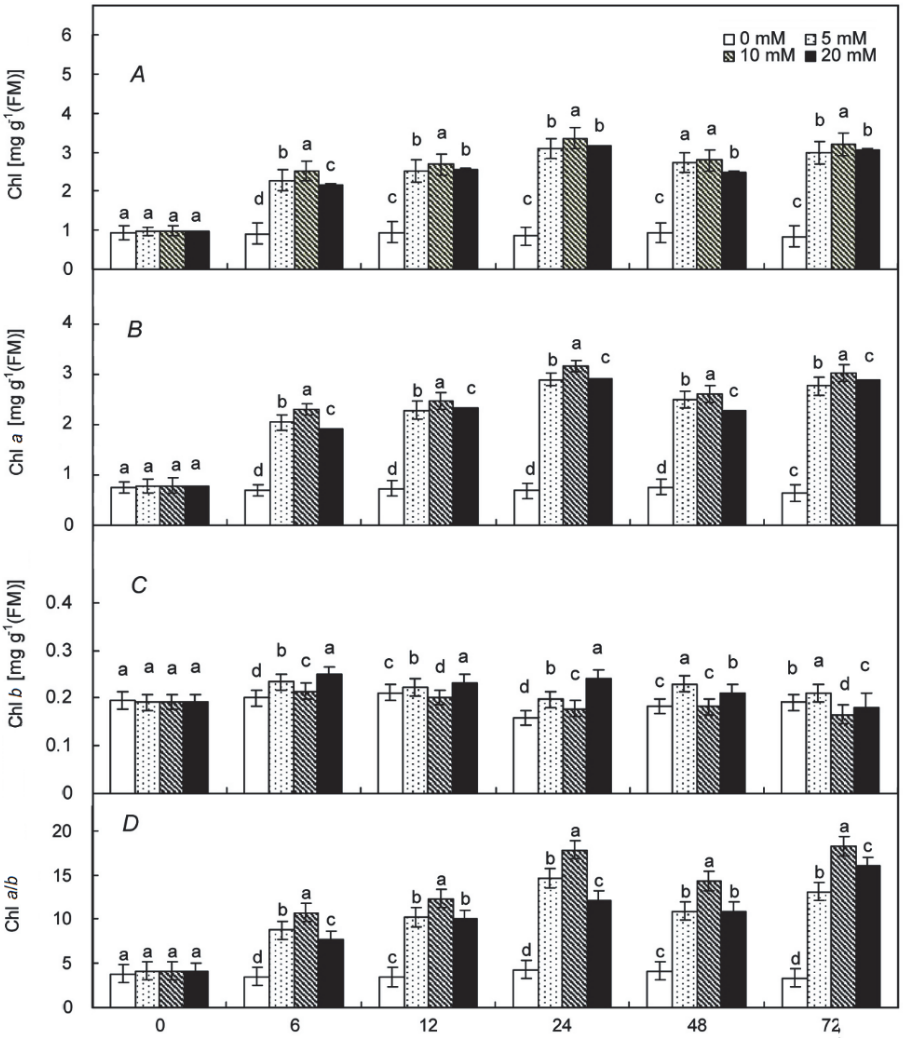

Fig. 4. Effect of drought stress and exogenous glycine on total Chl content (*A*), Chl *a* (*B*), Chl *b* (*C*), and Chl *a*/*b* (*D*) of *Chorispora bungeana* plantlets *in vitro* exposed to 0, 5, 10, and 20 mM glycine for 72 h. Values are the mean ± SD of at least three independent experiments. Chl – chlorophyll; FM – fresh mass; SD – standard deviation.

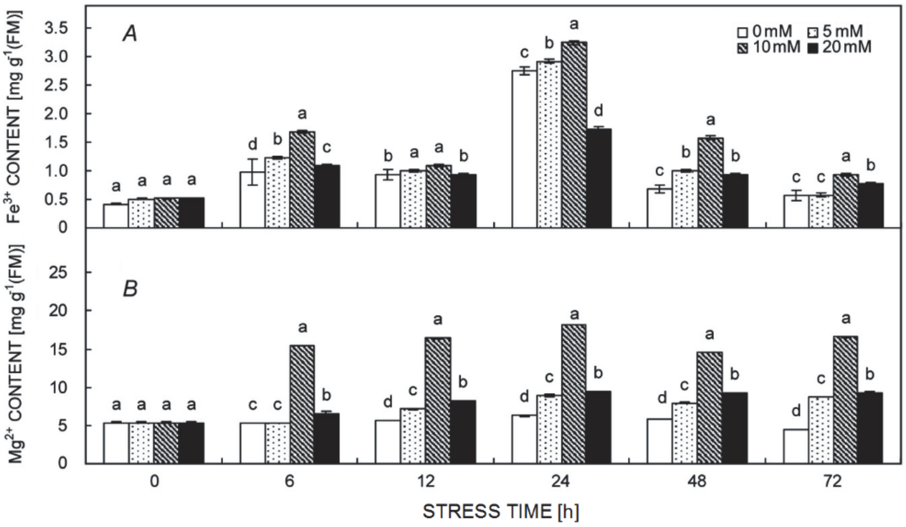

Fig. 5. Effect of drought stress and exogenous glycine on the content of $Fe^{3+}$ and $Mg^{2+}$ in *Chorispora bungeana* plantlets *in vitro* exposed to 0, 5, 10, and 20 mM glycine for 72 h. Values are the mean ± SD of at least three independent experiments. FM – fresh mass; SD – standard deviation.

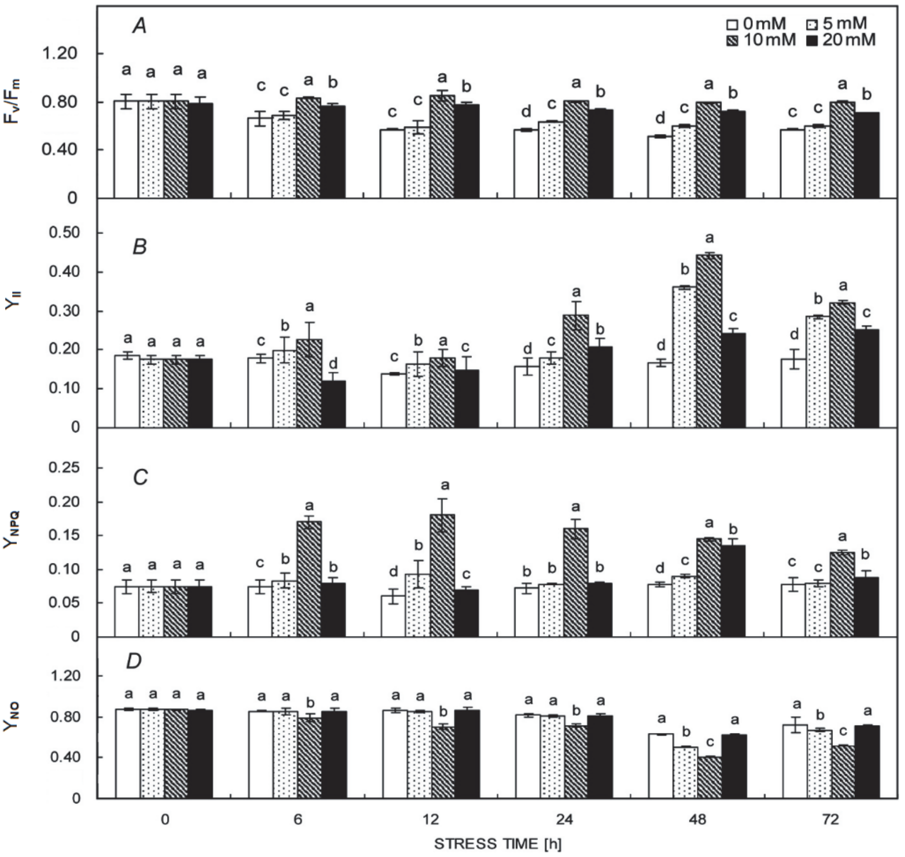

Fig. 6. Effect of drought stress and exogenous glycine on the changes of $F_v/F_m$ (*A*), $Y_{II}$ (*B*), $Y_{NPQ}$ (*C*), and $Y_{NO}$ (*D*) in *Chorispora bungeana* seedlings *in vitro* exposed to 0, 5, 10, and 20 mM glycine for 72 h. Values are the mean ± SD of at least three independent experiments. $F_v/F_m$ – maximal quantum yield of PSII photochemistry. $Y_{II}$ – actual quantum yield of PSII photochemistry; $Y_{NPQ}$ – quantum yield of regulated energy dissipation; $Y_{NO}$ – quantum yield of nonregulated energy dissipation; SD – standard deviation.

**Chl fluorescence parameters**: The $F_v/F_m$ ratio can be considered a measure of the maximal quantum yield of PSII photochemistry (Guo *et al*. 2000), which reflects a possibility to utilize light energy for photosynthesis. $F_v/F_m$ was an important parameter in plants responding to external environment, which was used in studing the photosynthetic system and predicting the growth trend of the plant (Ying *et al*. 2000). The $F_v/F_m$ values, between 0.8 and 0.85, show a very small change under normal growth conditions (Björkman *et al*. 1987). But this parameter prominently decreased when the plants were subjected to abiotic stresses, high light or genetic mutation (Lichtenthaler *et al*. 1997). $Y_{II}$, the actual quantum yield of PSII photochemistry for the light-adapted state, reflects a degree of open PSII (estimated by $q_P$) and maximal quantum yield of PSII photochemistry for the light-adapted state (estimated by $F_v'/F_m'$). $Y_{II}$ has a close relationship to carbon assimilation and fixation; $Y_{II}$ affects $CO_2$

assimilation in plants (Souza *et al.* 2004).

In our experiment, there were little changes under 5% PEG-6000 stress compared with control. The $F_v/F_m$ decreased due to 20 and 40% PEG-6000 drought stress, as well as $Y_{II}$, which was distinctly reduced. This suggested that higher concentrations of PEG-6000 damaged PSII. It illustrated that severe stress probably impaired the function of PSII and the regulation ability of *C. bungeana*.

The quantum yield of regulated energy dissipation, $Y_{NPQ}$, is a protective mechanism, which maintains a high oxidative state of primary electron acceptors that accept PSII, reducing the probability of photosynthetic damage (Badger *et al.* 2000). Nonregulated energy dissipation, $Y_{NO}$, is the dissipation which is always present. Our experiment suggested that *C. bungeana* might possess the ability to regulate and cope with drought caused by the low concentration of PEG-6000. However, the photosynthetic apparatus of *C. bungeana* was damaged by the osmotic stress of the high concentration of PEG-6000 so that the photosynthesis of *C. bungeana* was inhibited. As a result, photosynthetic electron transport was inhibited and the photosynthesis or the $CO_2$ assimilation rates in leaves of *C. bungeana* were affected.

On the contrary, adding an appropriate concentration of glycine might protect the function of PSII and promote the photochemical activity of PSII and cyclic photosynthetic phosphorylation, which raises the leaf photosynthetic rate, when *C. bungeana* is subjected to drought stress.

In conclusion, we found that the contents of chlorophylls and ions as well as parameters of chlorophyll fluorescence were affected by different degree of drought stress. The parameters were obviously lowered under moderate and severe drought stress. Thus, we infered that chlorophyll synthesis and photosynthesis were suppressed. The chlorophyll, magnesium and iron ions contents, $F_v/F_m$, $Y_{II}$, and $Y_{NPQ}$ also increased under drought stress with 5 and 10 mM exogenous glycine. But there was a downward trend with 20 mM exogenous glycine under drought stress. Nonregulated energy dissipation, $Y_{NO}$, which can reflect the extent of light damage, decreased in all repetitions. So based on this fact, we hypothesize that glycine could relieve the drought stress, but the high concentration of glycine was not the main factor driving the growth of plants.

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